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Migraines in Mice?

Minireview

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Recently, the ion channel mutations causing both familial hemiplegic migraine (FHM) and episodic ataxia type-2 (EA-2) were identified as defects in the human voltage-dependent calcium channel α_{1A} subunit (Ophoff et al., 1996). Simultaneous with the identification of the FHM/EA-2 gene defects, the mutations underlying the tottering and leaner mouse phenotypes were identified as defects in the murine voltage-dependent calcium channel α_{1A} subunit (Fletcher et al., 1996). Because these genes are homologs, the mouse mutants tottering and leaner may be instrumental in unraveling the pathogenesis of FHM and EA-2.

Inherited ion channel mutations or "channelopathies" are the cause of several neurologic disorders in humans. Hypokalemic periodic paralysis, which is characterized by intermittent weakness, results from a calcium channel mutation (Ptacek et al., 1994), whereas hyperkalemic periodic paralysis is caused by a sodium channel mutation (Cummins et al., 1993). Episodic ataxia type 1 is produced by a point mutation in a potassium channel (Browne et al., 1994). The distinguishing phenotypic feature of these channelopathies is the transient nature of the neurologic dysfunctions which are characterized by paroxysmal episodes of abnormal motor control and/or muscle tone.

Both FHM and EA-2 patients also exhibit paroxysmal neurologic dysfunction typical of the channelopathies (Griggs and Nutt, 1995). FHM is characterized by brief periods of hemiparesis or weakness accompanied by migraine headaches; although the patients generally recover function, the hemiparesis can occur for hours, far outlasting the migraine. Further, cerebellar atrophy has also been noted in FHM patients. In contrast, EA-2 is associated with attacks of ataxia that are precipitated by stress or exercise; these prolonged episodes can last more than a day. EA-2 patients are essentially normal between attacks but some EA-2 patients may also exhibit cerebellar atrophy. Although in retrospect, these two disorders appear to belong to a phenotypic continuum, they are unique clinical entities which few suspected were the result of mutations within the same gene until EA-2 was mapped to the same interval as FHM.

The tottering (*tg*) mutation results in spike and wave discharges, mild ataxia, and intermittent convulsions. These mutants are characterized by spontaneous behavioral arrest associated with synchronous, bilateral cortical polyspike discharges that last from 0.3–10 seconds, similar to those that occur in human absence epilepsy (Noebels and Sidman, 1979). Tottering mice also exhibit convulsions characterized by 20–40 minutes of coordinated spasms and jerks of the limbs, trunk, and face; similar to EA-2, these paroxysmal episodes are precipitated by stress. The leaner (tg^{1a}) mutation is an allele of tottering that causes profound chronic ataxia associated with pervasive Purkinje and granule cell loss throughout the anterior cerebellum and reduced cerebellar size. Like tottering mice, spike and wave discharges have been detected in leaner mice, but the paroxysmal convulsions are never observed. Because of the dramatically different phenotypes, *tg* and tg^{1a} were initially characterized as mutations of different genes (Sidman et al., 1965). However, like FHM and EA-2, genetic linkage and complementation studies demonstrated that *tg* and tg^{1a} are different alleles of a single genetic locus.

Calcium Channel α_{1A} Subunits

Defects in the voltage-dependent calcium channel α_{1A} subunit have been identified in FHM and EA-2 in humans and in tottering and leaner mice. Voltage-dependent calcium channels regulate several biologic functions including neurotransmitter release and excitability by controlling the flux of calcium. These channels are characterized by voltage-sensitive activation in response to depolarization resulting in the selective increase in calcium flux into the cell. The channel is then inactivated and returned to its resting state. The kinetics and voltage dependence of activation and inactivation define the specific calcium channel subtype as L, N, P/Q, or R (for reviews see Catterall, 1995; Perez-Reyes and Schneider, 1995; Wheeler et al., 1995). Calcium channels are composed of five subunits (α_1, α_2, β, γ, and δ); however, the α_1 subunit alone is sufficient to form the structural channel and confer voltage sensitivity. The calcium channel α_1 subunit topology is very similar to the α_1 subunit of voltage-dependent sodium channels and is characterized by four repeated units or domains (I–IV) of six alpha helical membrane spanning segments (S1–S6; Figure 1). These four domains interact to form the pore, confer ion selectivity, and regulate voltage-sensitivity, while the α_2, β, γ, and δ subunits modify these characteristics.

At least six genes (A, B, C, D, E, and S) comprise the α_1 subunit family, each with unique kinetic and pharmacologic properties. The α_{1A} subunit encodes P- and Q-type calcium channels which were originally identified in cerebellar Purkinje cells (Llinas et al., 1989) and granule cells (Zhang et al., 1993), respectively. Both P- and Q-type channels are high-voltage activated calcium channels. However, these channels can be distinguished by their pharmacologic properties and inactivation kinetics; P-type channels do not inactivate during a 1 second depolarizing pulse whereas Q-type channels rapidly inactivate. Differences in P- and Q-type inactivation kinetics may be determined by α_{1A} subunit splice variants (Snutch et al., 1991), suggesting that alterations in heteronuclear RNA processing caused by a mutation, such as those observed in EA-2 and tg^{1a}, could have a profound effect on the tissue-specific expression of calcium channel subtypes.

Are the Human and Mouse Calcium Channel α_{1A} Subunits Orthologs?

The mouse and human calcium channel α_{1A} subunits are encoded by homologous genes. The predicted amino

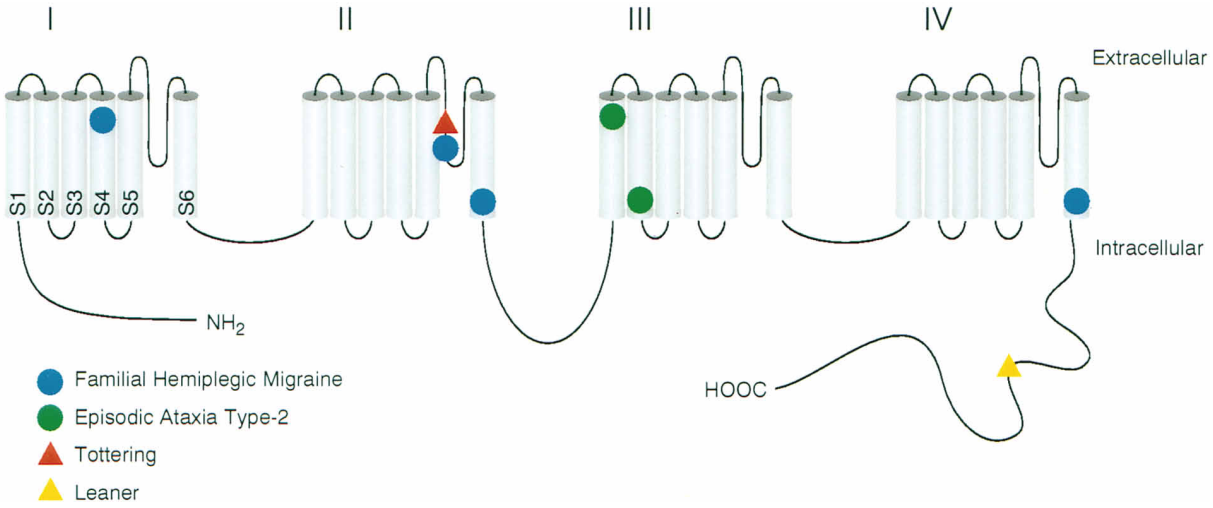

Figure 1. Membrane Topology of the Calcium Channel α_{1A} Subunit with Approximate Positions of Human and Murine Mutations Identified to Date

See text for details.

acid sequence of the mouse (GenBank U76716) and human (GenBank M64373) calcium channels α_{1A} subunit are 94% identical with 97% amino acid similarity. Furthermore, the transmembrane regions and the pore forming domains, which confer functional specificity, exhibit even greater (99%) identity. Differences between the sequences occur at the translational start site and in the intracellular loop between domains II and III. The translational start site in mouse is apparently 46 amino acid residues downstream of the predicted start site for both rat and human, which may be the result of alternative splicing. Sequence differences in the long intracellular loop spanning domains II and III may also result from splice variation as alternative splicing has been observed in this segment (Snutch et al., 1991). Despite these differences, the high degree of sequence identity between human and murine suggests these channels subserve similar functions.

The chromosomal position of the α_{1A} subunit gene is also consistent between human and mouse. Both tottering and leaner mutations have been mapped close to Lyl1 and Junb on mouse chromosome 8. This region of mouse chromosome 8 is syntenic with human chromosome 19p13 where both FHM and EA-2 have been mapped. The conservation of these chromosomal segments between mouse and human strongly suggests these genes are evolutionarily related. Furthermore, the distribution of the α_{1A} subunit mRNA is similar across species; rhesus monkey and mouse both express α_{1A} subunit mRNA at highest levels in the cerebellum with moderately high levels in the cortex and less transcript in the thalamus (Ophoff et al., 1996; Fletcher et al., 1996). Little or no α_{1A} subunit mRNA is detected outside the central nervous system. Limited analysis of the α_{1A} subunit distribution in human is consistent with these results demonstrating high levels of expression in the cerebellum (Volsen et al., 1995) and no expression detectable in lymphocytes. The sequence similarity, conservation of synteny, and tissue-specific mRNA distribution suggest that the human and murine α_{1A} subunit genes implicated in FHM/EA-2 and *tg*/*tg*[1a] are orthologs.

α_{1A} *Subunit Mutations*

A total of eight different mutations have been identified in the α_{1A} subunit (Figure 1). Four different mutations that cause FHM have been identified in five unrelated families; all are missense point mutations. Two of these mutations affect the S6 transmembrane alpha helices, regions that may form the intracellular mouth of the ion pore (Catterall, 1995). Another FHM mutation missense mutation is located very close to the tottering point mutation in the hairpin loop between S5 and S6. This is the pore-forming domain of the calcium channel, which is thought to confer ion-selectivity to the channel (Yang et al, 1993). Thus, more than half the mutations so far identified affect the pore itself and may compromise ion selectivity or permeability. The fourth FHM mutation occurs in the voltage sensor S4 segment of domain I suggesting that the channel response to depolarization may be compromised. Unlike the FHM and tottering mutations, the mutations underlying EA-2 and leaner cause gross disruptions in the α_{1A} subunit. Mutations in splice sites near the 3′ end of the gene have been identified in an EA-2 patient and in leaner mice. Additionally, a single nucleotide deletion in the S1 segment of domain III encodes a premature stop in the α_{1A} subunit in another EA-2 patient. The EA-2 and leaner mutations likely produce peptides that are not functional. The mutations identified to date appear to sort by phenotypic expression with alterations of a single residue representing FHM and tottering while more profound defects in the peptide structure define EA-2 and leaner.

Ion Channels and Paroxysmal Events

Altered ion permeability would clearly affect intercellular signaling and may precipitate the paroxysmal episodes. But how can a channelopathy generate a prolonged paroxysmal event? The episodic events observed in FHM/EA-2 and tottering/leaner generally last for tens of minutes to tens of hours; the shortest events, the spike and wave discharges observed in tottering and leaner mice, last for several seconds. By contrast, calcium channel activation and inactivation occur on the order of milliseconds to at most seconds in vivo. It is difficult

to directly reconcile the prolonged phenotypic events with the molecular events as even mutant channels are unlikely to sustain abnormal activity for such extended periods. Both exocytotic release and neuronal electrical properties are regulated by calcium channels. Therefore, given the extended expression of the episodic phenotypes, these prolonged neurologic events are likely to involve abnormal polysynaptic signaling generated or sustained by the disrupted neurotransmission.

These mouse mutants may serve as direct animal models of FHM and/or EA-2. The close proximity of the tottering mutation to one of the FHM mutations suggests the functional calcium channel defect may correspond in both human and mouse. Furthermore, while the phenotypes expressed in man and mice are not identical, they are very similar, mimicking both the paroxysmal nature of the disorder and the cerebellar atrophy. Of course, it is not reasonable to expect direct phenotypic correspondence between mouse and human even where the genetic defects are identical; this is especially true for FHM since it is not clear that mice (tottering or otherwise) experience migraine headaches. Nonetheless, these mice may provide a model for understanding the relationship between migraine headaches and ion channel misregulation. The genotypic and phenotypic similarities between the channelopathies in humans and the tottering mouse mutants suggest that tottering mice will be extremely useful for determining how a channelopathy can generate prolonged paroxysmal neurologic dysfunction in humans.

Selected Reading

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